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19. ABSTRACT (Continue on reverse if necessary and identify by block number) To study the electronic properties of double-stranded DNA as to determining whether this macromolecule can support electron transport processes. This pertains to possibly utilizing the base sequence and secondary structure of DNA as a matrix for developing molecular level electronic components. Toward these goals, we have studied the anisotropic electronic properties of DNA single crystals using reflectance spectroscopy and studied the interactions of transitions metals with double-stranded DNA by X-ray diffraction. We have also synthesized a number of porphyrin and acridine modified DNA molecules, and assembled a photoflash photolysis apparatus for direct study of electron transfer through DNA. In related work, we have shown that the propensity for DNA to adopt specific double helical conformations can be predicted from calculations of solvent accessible surfaces. From this, we were able to obtain diffraction quality single crystals of DNA oligomers in a predictive manner.																
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Summary of Work

ACCOMPLISHMENTS (3 years): We have successfully synthesized a number of porphyrin and acridine derivatives for direct measurement of electron transport through DNA. We are currently determining the steady-state reduction potentials of the modified DNAs to assess the utility of these as photoinducible electron donors and acceptors. We have also completed and tested the flash photolysis apparatus for the direct absorption and emission measurements of electron transfer rates. The primary drawbacks in the progress of this project have been the difficulty in the synthesis of the modified DNAs, and in assembling a working flash photolysis apparatus. Both problems have been solved or circumvented and we are now ready to perform the actual electron transfer measurements.

In related studies, the electronic properties of DNA single crystals were recorded (Ho, et al., 1990), and compared for d(CG) and d(UA) base pairs in Z-DNA by recording the polarized reflectance spectra of d(m⁵CG)₃ and d(m⁵CGUAm⁵CG) single crystals. These studies show that the electronic coupling between the aromatic bases of DNA duplexes are highly sequence specific. The sequence containing only d(CG) base pairs showed much greater resolution of the two major π - π^* transitions that are characteristic of the Z-conformation. We are currently in the process of comparing the polarized absorbance spectra of a number of other DNA sequences and conformations. In one study, we plan to use the UV absorption spectrum and its linear dichroism of a DNA sequence in an unsolved crystal to determine the conformation and orientation of the oligonucleotide in the unit cell. This would be an attempt to utilize the spectroscopic properties of a crystal to help in solving the phase problem associated with determining the structure of the DNA.

We have solved the structures of two oligonucleotide sequences in the presence of copper(II) ions, and shown that this metal binds in a covalent manner to the guanine bases of double stranded DNA (Kagawa, et al, 1991). The binding is base specific. Only purines are bound and, of the purine bases, only guanines are consistently modified (Geierstranger, et al, 1991). The binding of copper (II) to adenine bases must be facilitated by additional intermolecular interactions, and would not be expected to occur with DNA in free solution. Thus double stranded DNA crystals can be modified, or 'doped', in a base specific manner to affect the electronic properties of the crystal.

In other related work, we have developed and characterized a theoretical criteria for predicting the relative stability of various DNA sequences as Z-DNA in solution and, from this, predicting the solution conditions for the crystallizing hexanucleotides as Z-DNA (Ho, et al, 1991). The method uses solvent accessible surface calculations to estimate the relative stability of the hexanucleotides in the Z-form versus the B- and single-stranded conformations (Kagawa, et al, 1989). This lead to a prediction for the driving force required to induce formation of the left-handed conformation in solution and, when coupled with information on the intrinsic solubility of the conformation, leads to a prediction for how to crystallize Z-DNA. We applied this to the sequences d(m⁵CGUAm⁵CG), where m⁵C is the 5-methylated cytosine and U is deoxyuridine (Zhou and Ho, 1990), and d(CICGCG), where the base I is deoxyinosine, and found that the method predicted exactly the conditions at which Z-DNA crystals formed. When we examined the energetics for the packing of Z-DNA hexamers in the crystal lattice, we found that the lowest energy packing of the

hexanucleotides was that observed for the actual crystals. The energy for packing resulted from van der Waal's contacts that overcome the inherent conformational entropy and electrostatic repulsion of the DNA in the crystal.

SIGNIFICANCE: The ability to predict the crystallization of Z-DNA hexanucleotides is significant in that we can now consider the process of crystallizing biologically interesting macromolecules as a science rather than a random set of events. This will have applications towards the ability to crystallize other oligonucleotides in different conformations, and relates to the general process of macromolecular crystallization for x-ray diffraction studies.

The base dependance of the polarized reflectance spectra from of DNA in single crystals shows that the electronic properties of the DNA polymer are attenuated by base composition.

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PUBLICATIONS AND REPORTS FROM WORK SUPPORTED BY THE ONR (3 years):

- Kagawa, T.F., Stoddard, D., Zhou, G., and Ho, P.S. (1989) "A quantitative analysis of DNA secondary structure from solvent accessible surfaces: The B- to Z-DNA transition as a model", *Biochemistry*, **28**, 6642-6651.
- Zhou, G., and Ho, P.S. (1990) "Stabilization of Z-DNA by demethylation of thymine bases: 1.3 Å single crystal structure of d(m⁵CGUAm⁵CG)", *Biochemistry*, **29**, 7229-7236.
- Ho, P.S., Zhou, G., and Clark, L.B. (1990), "Polarized electronic spectra of Z-DNA single crystals", *Biopolymers*, **30**, 151-163.
- Green, S.A., Simpson, D.J., Zhou, G., Ho, P.S., and Blough, N.V. (1990), "Intramolecular quenching of excited singlet states by stable nitroxyl radicals", *J. Am. Chem. Soc.*, **112**, 7337-7346.
- Daniels, M., Hart, L.P., Ho, P.S., Ballini, J.-P., and Vigny, P. (1990), "Time-resolved spectroscopy of the intrinsic fluorescence of nucleic acid species", *SPIE Proc.*, **1204**, 304-313.
- Kagawa, T.F., Wang, A.H.-J., Stoddard, D., and Ho, P.S. (1991), "Covalent modification of guanine bases in DNA by copper (II) ions: 1.2 Å crystal Z-DNA structure of CuCl₂ soaked d(CG)₃", in press, *J. Biol. Chem.*
- Geierstranger, B., Kagawa, T.F., Quigley, G.J., and Ho, P.S. (1991), "Sequence specific modification of purine bases in DNA by copper (II) ions: 1.3 Å crystal Z-DNA structure of CuCl₂ soaked d(m⁵CGUAm⁵CG)", in press, *J. Biol. Chem.*
- Ho, P.S., Kagawa, T.F., Tseng, K., Schroth, G., and Zhou, G. (1991), "Prediction of a crystallization pathway for Z-DNA hexanucleotides", Submitted for publication.
- Tseng, K., Kagawa, T.F., and Ho, P.S. "Effect of minor groove substituent groups on the stability of Z-DNA", in preparation.

LECTURES AND ABSTRACTS ON ONR FUNDED PROJECTS (3 YEARS):

- Hunter College, the City University of New York, New York, NY, September 8, 1989: 'The effect of hydration on DNA structure', invited lecture.
- Reed College, Portland, OR, October 25, 1989: 'Water and DNA structure: The B- to Z-DNA transition as a model', invited lecture.
- ONR Contractors' Meeting, Belmont House, MD, November 12, 1989: 'DNA mediated electron transfer', invited lecture.
- 35th annual Biophysical Society Meeting in San Francisco, CA, February 26, 1991 'Substituent effects on DNA stability: A solvent free energy analysis', contributed lecture.
- Oregon Graduate Institute of Science and Technology, Beaverton, OR, March 10, 1991: 'Copper DNA interactions', invited lecture.
- Sixth Conversation in the Discipline of Biomolecular Stereodynamics, SUNY at Albany, June 6-10, 1989: 'The effect of hydration on DNA structure', Abstract.
- American Crystallographic Association Annual Meeting, Seattle, WA, July 23-29, 1989: 'Effect of hydration on DNA structure', contributed abstract.
- American Crystallographic Association Annual Meeting, Seattle, WA, July 23-29, 1989: 'The effect of methylation on the stability of d(TA) base pairs in Z-DNA', contributed abstract.

AWARDS AND RECOGNITION TO PARTICIPANTS AND PRINCIPAL INVESTIGATOR IN
THIS PROJECT:

Guangwen Zhou, Linus Pauling Award for student poster, American
Crystallographic Association Annual Meeting, Seattle, WA, July 23-29,
1989.

P. Shing Ho: American Cancer Society Junior Faculty Research Award.

STUDENT TRAINING:

<u>Student:</u>	<u>Nationality:</u>	<u>M/F:</u>	<u>Degree:</u>
Todd Kagawa	US citizen	Male	Ph.D. candidate
Guangwen Zhou	PRC	Male	Ph.D. candidate
Bernhard Geierstranger	Germany	Male	M.S., 1990

Patents: None filed or pending